CLAIMS

What is claimed as the invention is:

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1. A differentiated cell population that can be cultured *in vitro* culture, obtained by differentiating primate pluripotent stem (pPS) cells, wherein at least about 60% of the cells in the population have at least three of the following characteristics:
    - antibody-detectable expression of α_1-antitrypsin (AAT);
    - antibody-detectable expression of albumin;
    - absence of antibody-detectable expression of α-fetoprotein;
    - RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
    - evidence of glycogen storage;
    - evidence of cytochrome p450 activity;
    - evidence of glucose-6-phosphatase activity; and
    - the morphological features of hepatocytes.

2. The differentiated cell population of claim 1, ~~wherein~~ at least about 60% of the cells have at least five of said characteristics.

3. The differentiated cell population of claim 1, wherein at least about 80% of the cells have at least seven of said characteristics.

4. The differentiated cell population of claim 1, wherein the pPS cells from which the differentiated cells have been differentiated are human embryonic stem (hES) cells.

5. The differentiated cell population of claim 1, which has been genetically altered to express telomerase at an elevated level.

6. The differentiated cell population of claim 1, obtained by culturing the pPS cells in a growth environment that comprises a hepatocyte differentiating agent.

7. The differentiated cell population of claim 6, wherein the hepatocyte differentiating agent is n-butyric acid.

8. The differentiated cell population of claim 1, obtained by culturing the pPS cells in a growth environment that comprises an extracellular matrix.

9. The differentiated cell population of claim 1, obtained by culturing the pPS cells in a growth environment that comprises one or more hepatocyte maturation factors.

10. The differentiated cell population of claim 9, wherein at least one of the hepatocyte maturation factors is either:

a) an organic solvent selected from the list consisting of dimethyl sulfoxide (DMSO), dimethylacetamide (DMA); hexmethylene bisacetamide, and other polymethylene bisacetamides; or

b) a cytokine or hormone selected from the group consisting of glucocorticoids, epidermal growth factor (EGF), insulin, TGF-α, TGF-β, fibroblast growth factor (FGF), heparin, and hepatocyte growth factor (HGF), IL-1, IL-6, IGF-I, IGF-II, and HBGF-1.

11. A differentiated cell population according to claim 1 in a composition essentially free of feeder cells, extracellular matrix, hepatocyte differentiation agents, and hepatocyte maturation factors.

12. A differentiated cell having at least three of the characteristics listed in claim 1, which is either harvested from the differentiated cell population according to claim 1, or is the progeny of such a cell.

13. A differentiated cell produced by providing a human pluripotent stem (hPS) cells in a growth environment essentially free of feeder cells; culturing the hPS cells in a medium containing a hepatocyte differentiation agent under conditions that produce a cell population enriched for cells with characteristic features of hepatocytes; and subsequently harvesting a cell having these features from the enriched cell population.

14. A method of treating human pluripotent stem (hPS) cells to obtain differentiated cells that can be cultured *in vitro*, comprising:

a) providing a culture of human pluripotent stem cells;

b) culturing the cells on a substrate in a culture medium containing a hepatocyte differentiation agent under conditions that lead to enrichment of the differentiated cells.

15. The method of claim 14, wherein the culture of hPS cells is produced by culturing the cells under conditions whereby embryoid bodies form; plating the embryoid bodies on an extracellular matrix; adding the hepatocyte differentiation agent to the medium simultaneously or subsequently to the plating of the embryoid bodies; and then culturing the plated cells with the hepatocyte differentiation agent under conditions that permit enrichment of the differentiated cells.

16. The method of claim 14, wherein the hepatocyte differentiation agent is added to a culture of hPS cells comprising an extracellular matrix but essentially free of feeder cells; and then culturing the hPS cells with the hepatocyte differentiation agent under conditions that permit enrichment of the differentiated cells.

17. The method of claim 16, wherein the extracellular matrix comprises laminin, collagen, or matrix from Engelbreth-Holm-Swarm cells.

18. The method of claim 14, wherein the hepatocyte differentiation agent is n-butyric acid.

19. The method of claim 14, further comprising culturing the cells with one or more hepatocyte maturation factors, simultaneously or sequentially to the culturing with the hepatocyte differentiation agent.

20. The method of claim 19, wherein at least one of the hepatocyte maturation factors is either:

a) an organic solvent selected from the list consisting of dimethyl sulfoxide (DMSO), dimethylacetamide (DMA); hexmethylene bisacetamide, and other polymethylene bisacetamides; or

b) a cytokine or hormone selected from the group consisting of glucocorticoids, epidermal growth factor (EGF), insulin, TGF-α, TGF-β, fibroblast growth factor (FGF), heparin, and hepatocyte growth factor (HGF), IL-1, IL-6, IGF-I, IGF-II, and HBGF-1.

21. A differentiated cell, produced according to the method of claim 14, or the progeny of such a cell.

22. The differentiated cell of claim 21, having at least three of the following characteristics:

- antibody-detectable expression of α_1-antitrypsin (AAT);
- antibody-detectable expression of albumin;
- absence of antibody-detectable expression of α-fetoprotein;
- RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
- evidence of glycogen storage;
- evidence of cytochrome p450 activity;
- evidence of glucose-6-phosphatase activity; and
- the morphological features of hepatocytes.

23. A method of screening a compound for hepatocellular toxicity or modulation, comprising contacting a differentiated cell according to claim 12 with the compound, determining any phenotypic or metabolic changes in the cell that result from contact with the compound, and correlating the change with hepatocellular toxicity or modulation.

24. A method of screening a compound for hepatocellular toxicity or modulation, comprising contacting a differentiated cell according to claim 21 with the compound, determining any phenotypic or metabolic changes in the cell that result from contact with the compound, and correlating the change with hepatocellular toxicity or modulation.

25. A method of detoxifying a fluid, comprising contacting the differentiated cell according to claim 12 with the fluid under conditions that permit the cell to remove or modify a toxin in the fluid.

26. A liver support device, in which a differentiated cell according to claim 12 is situated to detoxify blood that is flowing from a patient and then returned to the patient after being detoxified by the device.

27. A method of reconstituting or supplementing hepatocellular function in an individual, comprising administering into the abdominal cavity of the individual a plurality of differentiated cells according to claim 12.

28. A method of reconstituting or supplementing hepatocellular function in an individual, comprising connecting the individual to the liver support device of claim 26 in such a manner that their blood circulates through and is detoxified by the device.

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